solution, a smaller molecule (1127 MW fluorescently labeled ATTO633-ATP) is restricted more than a larger one (10000 MW Alexa647-dextran). We attempt to provide a resolution to this counterintuitive result by applying a quantitative stochastic model of diffusion where diffusion obstacles are formed by a 3D lattice of permeable barriers. The model is able to reproduce experimental results with periodic intracellular barriers situated 1 μm apart, having very low permeabilities and a small effect of molecular crowding in volumes between the barriers. The distances we obtained match those of several intracellular structures in both longitudinal and transverse directions. Such intracellular structuring restricts diffusion of molecules of energy metabolism, reactive oxygen species and apoptotic signals, enacting a significant role in normally functioning cardiomyocytes as well as in pathological conditions of the heart.

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Role of Vinculin in Ventricular Wall Function

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Vinculin (Vcl) is a ubiquitously expressed cytoskeletal protein that localizes at cell adhesion sites in cardiomyocytes. It is a key component in the costamere, which anchors the sarcomeric cytoskeleton to the extracellular matrix via integrins. It has been previously shown that it plays a significant role in mechanotransduction and may affect myocardial mechanical function in a directional-dependent manner. To determine the role of Vcl in regional wall mechanics, cardiac MRI tagging was performed in a cardiac-myocyte-specific vinculin knockout (VclKO) mouse model revealing significant decreases in systolic sheet-normal shear strain ($P<0.05$) and systolic sheet strain ($P<0.05$) in KO mice compared with littermate wildtype (WT) controls. In addition, measurements in isolated papillary muscles in heterozygous global vinculin knockout mice showed no difference in isometric fiber tension development consistent with no change in systolic fiber strain in vivo. A finite element model of ventricular mechanics suggested an increase in transverse systolic stress development may explain these observations. We hypothesized that this may be due to an increase in myofilament lattice spacing in the VclKO. Lattice spacing and sarcomere length were measured from optical diffraction patterns generated from FFTs of electron micrographs of VclKO and control hearts. The center-to-center spacing between myosin filaments was 37.6 ± 2.55 nm in barium-contracted VclKO hearts (n=3) compared with 32.9 ± 2.37 nm in WT controls (n=3). In hearts fixed at zero load, this spacing averaged 33.5 ± 1.35 nm in VclKO (n=3) vs. 30.8 ± 0.68 nm in WT (n=3). These results along with sarcomere length measurements suggest Vcl can mediate myofilament architecture and hence directional-dependent systolic force generation. We hypothesize that increasing lattice spacing alters the crossbridge binding angle of myosin heads, increasing the transverse force that they generate. The resulting increase in transverse myofiber stiffness during systole decreases systolic sheet strains.

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Differential Regulation of Glycolysis in Neonatal Versus Adult Cardiac Myocytes: The Role of Hexokinase Isoforms

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In cardiac cells there is a dramatic switch at birth in metabolism from glycolysis to fatty acid utilization. This switch is paralleled by a switch between the two isoforms of hexokinase, HK1 and HK2, which phosphorylate glucose as the first step in glycolysis. HKI predominates in neonatal cardiac myocytes (NRVM) and HKII in adult cardiac myocytes. Using real time optical methods in isolated cells to study the function and localization of HKs, we found that NRVMs have a much higher glycolytic activity than adult cells. This increased activity is accompanied by stronger HK's interaction with mitochondria as judged by fusion of HK's with fluorescent proteins. In both cell types, all HKI is bound to mitochondria, while HKII distributes between the mitochondria and cytosol. Removal of extracellular glucose displaces HKII from mitochondria in adult cells, but not in NRVMs. The glycolytic inhibitor iodoacetate, which raises intracellular G6P levels, displaced HKII from the mitochondria in both adult and NRVMs but had no effect on HKI distribution. Based on these results, we attribute elevated glycolytic activity of NRVMs to their preferential expression HKI, whose intrinsically higher binding affinity for mitochondria directs glucose and G6P to glycolysis instead of glycogen formation. This in turn lowers G6P levels, facilitating the interaction of HKII with mitochondria. Since HK binding to mitochondria facilitates cardioprotection, this factor may also account, at least in part, for the greater resistance of NRVMs to ischemia/reperfusion injury compared to adult cardiac myocytes.

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Spatial Localisation and Function of pH-Regulatory Transporters in the Rat Ventricular Myocyte

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The principal acid-extruding proteins in ventricular myocytes are Na^+/H^+ exchange (NHE1) and Na^+-HCO_3^- cotransport (NBC). We explored their spatial distribution and activity in enzymically isolated adult rat ventricular myocytes (ARVM). Confocal images of ARVM immunostained with Alexafluor 488-coupled antibodies (n=4 to 60) revealed strong expression for NHE1 at intercalated discs, with lower surface-sarcolemmal expression. For NBCe1 and n1, there was intense expression in the transverse tubules (t-tubules) and some at the sarcolemmal-surface.

We studied functional pH_i regulation in control and detubulated myocytes. Detubulation was achieved using transient exposure to 1.5M formamide, and was confirmed by a) large absence of t-tubule staining with 8-di-ANEPPS, b) reduced membrane capacitance (138 ± 11 pF; n=10 vs 236 ± 25 pF; n=14, $p<0.05$), c) reduced L-type Ca^{2+}-current amplitude (-2.5 ± 0.5 pA/pF; n=9 vs -5.0 ± 0.8pA/pF; n=8, $p<0.05$), and c) spatially non-uniform upstroke of electrically-evoked Ca^{2+}-transient. H^+-equivalent efflux on NBC was measured during whole-cell pH_i-recovery from an acid load (recorded with AM-loaded SNARF; $5\%CO_2/22mMHCO_3^-$ superfusates containing 30μM cariporide; flux=dpH_i/dt x intracellular buffering power). In detubulated cells, flux was reduced by ~40% over the pH_i range 6.55-6.85 (n=10, $p<0.05$). In contrast, H^+ efflux on NHE (Hepes-buffered Tyrode) was unaffected (n=9, NS).

In control ARVM, NHE1 activation produced spatial pH_i-gradients along the major and minor axis of the cell (confocal pH_i-imaging: 0.086 ± 0.028 and 0.050 ± 0.015 pH units respectively, n=4), whereas no pH_i-gradients were observed for comparable H^+-efflux through NBC.

We conclude that NHE1 and NBC transporters show differential spatial expression in ARVM. NBC has privileged access to pH control of the EC-coupling apparatus in the t-tubules, while NHE1 will dominate the proton-control of gap junctions at intercalated discs. The spatial trafficking of NHE and NBC suggests a local control of pH in different regions of the cell.

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O-Glcnacylation in the Long Isoform of ZASP in Human Heart Muscle

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ZASP (Z-band alternatively spliced PDZ-motif protein, Cypher, Oracle) is a protein on the Z-line of the sarcomere and exists in long and short isoforms. The short isoforms lack the three LIM domains at the C-terminus of the protein and it is suggested that the long and short isoforms may have distinct roles in cardiac function. The posttranslational modification *O*-GlcNAc was detected on ZASP in human cardiac myofibril extracts (the insoluble fraction of tissue homogenization) by enzymatic labelling, by attaching the unnatural sugar *N*-acetylgalactosamine (UDP-GalNAz) onto *O*-GlcNAc groups using a mutant enzyme (Y289L β1,4-galactosyltransferase) and coupling to it the tetramethylrhodamine (TAMRA) fluorescent tag. Intense fluorescence signal was detected in SDS-PAGE of one of the protein bands, at ~90 kDa, for all the 10 samples tested. The protein was identified to be ZASP by MALDI-TOF/TOF analysis, and it forms less than 0.5% of all proteins in the insoluble fraction as determined in Coomassie Blue stained gels. Western blotting of the same extracts using a polyclonal antibody to ZASP detected protein bands at ~80-90 kDa, ~40 kDa and ~30 kDa. Antibodies to *O*-GlcNAc (CTD110.6 and RL2) detected the same ~80-90 kDa bands as *O*-GlcNAcylated. ZASP is present in approximately the same amount in the soluble fraction of cardiac tissue homogenization. Western blotting of this soluble fraction shows only the ~80-90 kDa bands were detected by the ZASP and *O*-GlcNAc antibodies, but not bands of lower molecular weights. This study suggests that the *O*-GlcNAcylation is only present in the long but not the short isoforms of ZASP, giving the possibility that the *O*-GlcNAc site(s) is present in the LIM domains, important for maintaining Z-line integrity.